

# Peanuts, Peanut Oil, and Fat Free Peanut Flour Reduced Cardiovascular Disease Risk Factors and the Development of Atherosclerosis in Syrian Golden Hamsters

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**ABSTRACT:** Human clinical trials have demonstrated the cardiovascular protective properties of peanuts and peanut oil in decreasing total and low density lipoprotein cholesterol (LDL-C) without reducing high density lipoprotein cholesterol (HDL-C). The cardiovascular effects of the nonlipid portion of peanuts has not been evaluated even though that fraction contains arginine, flavonoids, folates, and other compounds that have been linked to cardiovascular health. The objective of this study was to evaluate the effects of fat free peanut flour (FFPF), peanuts, and peanut oil on cardiovascular disease (CVD) risk factors and the development of atherosclerosis in male Syrian golden hamsters. Each experimental diet group was fed a high fat, high cholesterol diet with various peanut components (FFPF, peanut oil, or peanuts) substituted for similar metabolic components in the control diet. Tissues were collected at week 0, 12, 18, and 24. Total plasma cholesterol (TPC), LDL-C, and HDL-C distributions were determined by high-performance gel filtration chromatography, while aortic total cholesterol (TC) and cholesteryl ester (CE) were determined by gas liquid chromatography. Peanuts, peanut oil, and FFPF diet groups had significantly ( $P < 0.05$ ) lower TPC, non-HDL-C than the control group beginning at about 12 wk and continuing through the 24-wk study. HDL-C was not significantly different among the diet groups. Peanut and peanut component diets retarded an increase in TC and CE. Because CE is an indicator of the development of atherosclerosis this study demonstrated that peanuts, peanut oil, and FFPF retarded the development of atherosclerosis in animals consuming an atherosclerosis inducing diet.

**Keywords:** atherosclerosis, cholesterol, hamsters, peanut flour, peanuts

## Introduction

Peanuts (*Arachis hypogaea*) are legumes but are generally considered as nuts. The weight of peanuts consumed per year in the United States is greater than all other nuts combined (Putnam and Allshouse 1999). Peanuts have a desirable fatty acid profile and are rich in vitamins, minerals and bioactive materials. They contain several known heart healthy nutrients including monounsaturated and polyunsaturated fatty acids, potassium, magnesium, copper niacin, arginine, fiber,  $\alpha$ -tocopherol, folates, phytosterols, and flavonoids. Peanut consumption has been associated with improved overall diet quality and nutrient profile (Kris-Etherton and others 1999b; Kerckhoffs and others 2002; Griel and others 2004).

The American Heart Assoc. (AHA) has indicated that cardiovascular disease (CVD) remains as the number one cause of death of Americans (Lloyd-Jones and others 2008). Peanuts and peanut oil have been demonstrated to reduce CVD risk and/or risk factors in epidemiological and clinical studies (Fraser and others 1992; Hu and others 1998; Kris-Etherton and others 1999a; Alper and Matetes 2003). However, published literature on the effect of the peanut

nonlipid component (fat free peanut flour [FFPF]) on CVD risk factors is lacking as is literature on the effects of peanuts and peanut oil on atherosclerosis.

The development of atherosclerosis is the result of changes in arterial walls. Cholesteryl ester (CE) concentration is a strong indicator of the development of atherosclerosis because it is one of the first metabolic compounds to occur at the intima in diseased arteries (St. Clair and others 1970). The CE concentration can increase as much as seventy times in atherosclerotic arteries compared to healthy arteries (St. Clair and others 1970; Day and Proudlock 1974). Metabolic changes that occur during the early stages of atherosclerosis development also include an increase in phospholipids and triacylglycerols; however, increases in those components are much lower in magnitude than CE (Brecher and Chobania 1974). Additionally, CE concentrations have been noted to decrease when a reduction in atherosclerotic plaque occurs (St. Clair and others 1972).

The major plasma cholesterol carrier in hamsters, fed a diet enriched with cholesterol and saturated fat for a prolonged period, changes to low density lipoprotein cholesterol (LDL-C) which is the major plasma cholesterol carrier in humans. Hamsters also closely resemble humans with respect to rates of hepatic cholesterol synthesis (Andersen and Cook 1986) and are thus responsive to high saturated fat, high cholesterol diets (Nistor and others 1987; Spady and Dietschy 1988). Hamsters, as all animal models, have some limitations but overall they are a good model for studying the effects of various diets on blood chemistry

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risk factors and development of diet induced atherosclerosis (Nistor and others 1987). Male hamsters are more often utilized than female hamsters to reduce the possibility that cardiovascular protective effects could be caused by estrogens and not components in the diet (Hamm and others 1983; Morise and others 2006). The objective of this study was to examine the potential protective effects of FFPF on CVD risk factors and the effects of FFPF, peanut oil and whole peanuts on the development of atherosclerosis in male Syrian golden hamsters.

## Materials and Methods

### Animal care

Total of 82 male Syrian golden hamsters, 6-wk-old and weighing between 80 and 100 g, were purchased from Harlan Inc. (Indianapolis, Ind., U.S.A.). Hamsters were housed in individual cages with NEPCO (Northeastern Products Corp.) wood chip bedding (Warrensburg, N.Y., U.S.A.), wire top hopper covers and isolator top lids at the Biological Research Facility (BRF) at North Carolina State Univ. (NCSU), Raleigh, N.C., U.S.A. The hamsters were maintained on a 12/12 h light/dark cycle in an environmentally controlled room. Total of 76 hamsters were randomly assigned to 1 of 4 diet groups before the start of the experiment. Hamsters were weighed weekly until age 15 wk and then twice monthly. All procedures were approved by the NCSU Animal Care and Use Committee.

### Diets

The 4 experimental diets were fed *ad libitum* with clean water. The control diet was a modification of the AIN-76A Clinton/Cybulsky Cholesterol Series semipurified diet for rodents prepared by TestDiet® (Richmond, Ind., U.S.A.). This diet formulation was designed to increase blood chemistry risk factors for CVD and induce atherosclerosis in rodents (Lichtman and others 1999). There were 3 experimental diet groups: FFPF, peanut oil, or whole peanuts. All experimental diets were adjusted to be isocaloric to the control diet by substituting peanut products for components with equal metabolizable energy (fat = 9 kcal/g, carbohydrate and protein = 4 kcal/g). A vitamin mix was added to all diets at 1.1% to insure basic nutritional needs for growth and health.

Commercially available roasted peanut products (whole peanuts, peanut oil, and peanut flour [12% fat]) were a gift from Golden Peanut Co. (Alpharetta, Ga., U.S.A.). The 3 types of peanut products used in the diets were collected from the same processing lot. The commercial 12% oil peanut flour was further commercially hexane extracted to less than 0.5% oil. Fatty acid analysis indicated that the peanuts processed for this study were a high oleic variety and contained approximately 80% oleic acid and approximately 4% linoleic acid. Although the fatty acid content of normal oleic peanuts is approximately 55% oleic acid and 30% linoleic acid, published studies indicate that both high oleic and normal oleic peanuts/oil produce the same beneficial cardiovascular effect on blood lipid profiles (Obyrne and others 1977).

Hamsters were fed the original commercial diet (Purina 5001) for 1 wk after arrival at the BRF and then experimental diets were substituted for the commercial diet incrementally during the next 5 d. The 24-wk study was initiated after all hamsters were consuming 100% of the assigned experimental diet.

### Peanut component analyses

Fatty acid composition was determined for the whole peanuts and peanut oil. Before fatty acid analysis, the whole peanuts were

ground in a Braun coffee mill (Gillette Inc., Boston, Mass., U.S.A.) and then mechanically pressed to remove oil.

Fatty acids were prepared for gas chromatograph (GC) analysis in triplicate as described by Bannon and others (1985). A Perkin Elmer GC equipped with a flame ionization detector (FID) and a SGE BPX70 column (30 m × 0.25 mm ID × 0.25 µm dry film, Phenomenex, Torrance, Calif., U.S.A.) was used and peak identifications were made by matching retention times with authentic fatty acid methyl ester standards. The standards used were Kel-Fim FAME-5 Standard purchased from Matreya, LLC. (Pleasant Gap, Pa., U.S.A.) and GLC-21A purchased from Nu-Check Standards (Elysian, Minn., U.S.A.).

Tocopherols were analyzed in peanuts and peanut oil using a modification of the methods of Hashim and others (1993). A Luna 5-µm Silica column, 250 mm length, 4.6 mm i.d. (Phenomenex, Torrance, Calif., U.S.A., Cat nr 00G-4274-E0), a flow rate of 1.4 mL/min, mobile phase of 1% 2-propanol in hexane along with a Waters 248 Dual Wavelength Absorbance Detector was used for the high-performance liquid chromatography (HPLC) analysis. The standards were purchased from Sigma Chemical (St. Louis, Mo., U.S.A.) and covered 5 orders of magnitude that bracketed all sample concentrations.

Protein content and amino acid profiles were determined in triplicate for whole peanuts and FFPF before they were sent to TestDiet® to manufacture the experimental diets. Total percent protein was calculated using the Dumas method of nitrogen analysis and a conversion factor of 5.46 after analysis on a 2400 CHN Elemental Analyzer (Perkin-Elmer Corp., Norwalk, Conn., U.S.A.). Amino acid analysis was conducted using a modification of the method published by Hagen and others (1989). The samples were hydrolyzed and then derivatized using AccQ-Floor™ reagent as outlined in the Waters' (Waters Corp., Milford, Mass., U.S.A.) manual (WAT052874, Rev 0). A Summit Model HPLC (Dionex Corp., Sunnyvale, Calif., U.S.A.) with a Waters Acc-QTag column (C<sub>18</sub>, 4µ, 150 mm × 3.9 mm) was used in the analyses of derivatives. In the HPLC analysis, Eluant A was an aqueous phosphate buffer plus triethylamine solution from (Waters Corp.) diluted with deionized water. Eluant B was acetonitrile diluted with deionized water (60 : 40, v : v). The samples were spiked with an internal standard, alpha-aminobutyric acid (Sigma Chemical Corp.). A mixed standard (Pierce Biotechnology Inc., Rockford, Ill., U.S.A.) that contained all amino acids except tryptophan was analyzed with sample aliquots to construct an analytical response curve over a range of 0.2 to 1 µg/mL. Tryptophan was determined by Summit HPLC (Dionex, Sunnyvale, Calif., U.S.A.) with a LiChrospher 100 RP-18 column (250 mm × 4.6 mm, 5 µm, Alltech Corp., Deerfield, Ill., U.S.A.) without any additional derivatization. The standard curve was determined with 1 to 100 ppm of L-tryptophan Authentic Standard (Pierce Biotechnology Inc.) in HPLC water.

### Diet analyses

Fatty acid composition of each diet was determined in triplicate. Diets were ground into a powder with a Braun coffee mill (Gillette Inc., Boston, Mass., U.S.A.) and moisture was measured according to AOAC 934.01 (Padmore 1990). Fat was extracted from the diets by AOAC 954.02 animal feed method (Helrich 1990) and the samples were analyzed on a Perkin Elmer GC equipped with an Autosampler XL (Waltham, Mass., U.S.A.) and a Restek, RT-2560 column (100 m × 0.25 mm ID × 0.2 µm dry film, Bellefonte, Pa., U.S.A.) (Ngeh-Ngwainbi and others 1997). A moisture correction factor was used in the calculation of percent of each fatty acid (Pomeranz and Meloan 1987). Tocopherol concentrations in each experimental diet were determined in triplicate.

## Plasma analyses

Hamsters were euthanized at wk 0, 12, 18, and 24. The hamsters were desanguinated by cardiac puncture and approximately 2 mL of each blood sample was anticoagulated in heparinized tubes (7% EDTA). Individual tubes were placed on ice as samples were collected and stored at  $-60^{\circ}\text{C}$  until analysis. Heparinized tubes were tempered to room temperature and then centrifuged at  $6000 \times g$  for 10 min. Serum was decanted into screw-cap vials and delivered to the Dept. of Pathology, Lipid Science Div. at the Wake Forest Univ. School of Medicine (Winston-Salem, N.C., U.S.A.) for cholesterol analyses. Total plasma cholesterol (TPC), very low density lipoprotein cholesterol (VLDL-C), LDL-C, and high density lipoprotein cholesterol (HDL-C) were determined by high-performance gel-filtration chromatography (HPGC) using a 0.9% saline solution with 0.01% EDTA and 0.01% azide at 0.4 mL/min and a Superose 6 10/300 column (GE Healthcare, Piscataway, N.J., U.S.A.) (Carroll and Rudel 1983). The column effluent was split and half was mixed with total cholesterol reagent (Cholesterol H/P, Roche Diagnostics, Nutley, N.J., U.S.A.) being pumped at 0.125 mL/min. The area percent of each cholesterol fraction (VLDL-C, LDL-C, and HDL-C) was determined by the chromatography software (Kieft and others 1991).

## Aortic cholesterol analyses

The heart and at least 3 mm of the aorta were removed from euthanized hamsters. The aorta was dissected from the heart and both were frozen at  $-60^{\circ}\text{C}$ . The aortas were tempered to  $20$  to  $22^{\circ}\text{C}$  in buffered saline solution and placed on the platform of a dissecting microscope for careful removal of all adventitia tissue. Aortas were stored in 10% neutral buffered formalin for subsequent processing.

Atherosclerotic development was quantified for each hamster in this study at wk 0, 12 and 18 as aortic CE concentration, measured as mg/g protein (PR). The 24 wk samples were lost to an initial, unsuccessful, Oil Red O staining necessary for microscopic

examination of atherosclerotic lesions. The wet weight of the aortas was recorded after being gently blotted to remove exterior formalin. Lipid was extracted from the aortas with chloroform-methanol, 2 : 1 (v : v) containing  $20.5\text{ }\mu\text{g}$  of 5- $\alpha$ -cholestane as an internal standard. The lipid extract was separated by filtration, dried under  $\text{N}_2$  at  $60^{\circ}\text{C}$ , and then dissolved in hexane. Analyses of unesterified and total cholesterol were carried out with 2 injections per sample on a DB 17 (15 m  $\times$  0.53 mm ID  $\times$  1  $\mu\text{m}$ ) GC column (J&W Scientific, Folsom, Calif., U.S.A.) at  $250^{\circ}\text{C}$  in a Hewlett-Packard 5890 GC equipped with a Hewlett-Packard 7673A (Hewlett-Packard Co. LP, Houston, Tex., U.S.A.) automatic injector using online column injection and FID. The calculation of CE was done by determining the difference between unesterified and total cholesterol, as measured before and after saponification and reextraction of the nonsaponifiable sterol into hexane. A conversion factor of 1.67 was used to mathematically determine the removal the fatty acids from esterified cholesterol. The tissue was then digested and dissolved in 1 N NaOH, and total protein was determined by the Lowry protein assay (Lowry and others 1951).

## Statistical methods

SAS software (Cary, N.C., U.S.A.) was used for all statistical evaluations. The plasma cholesterol and aortic cholesterol were analyzed as a 2-variable study and means separation was conducted to account for time and diet effects. The plasma cholesterol results had inhomogeneity variance requiring transformation. The log transformation of the plasma cholesterol measurements was used to correct for the variance. The log transformation of aortic TC and CE plus 1 was used for the end point of atherosclerosis because some measurements at time points were zero (Rao 1998). *P*-values  $< 0.05$  were considered significant.

## Results

Analyses were performed on all 4 experimental hamster diets which contained 0.5% cholesterol and 40% to 43% total fat (Table 1). The amino acid content of the whole peanuts and FFPF

**Table 1 – Composition of control and peanut component experimental diets.**

Ingredient	FFPF diet <sup>a</sup> composition, %	Peanut oil diet <sup>b</sup> composition, %	Peanut diet <sup>c</sup> composition, %	Control diet <sup>d</sup> composition, %
Corn starch	21.60	27.69	23.55	23.80
Fat free peanut flour	20.00	—	—	—
Peanut oil	—	20.00	—	—
Whole peanuts	—	—	20.00	—
Casein-vitamin free	10.36	21.95	16.44	22.46
Cocoa butter	16.20	0.00	11.50	17.40
Sucrose	10.40	10.10	9.50	12.69
Maltodextrin	6.50	6.00	7.00	7.97
Powdered cellulose	4.56	5.80	3.06	5.61
Soybean oil	2.65	1.84	1.84	2.81
Potassium citrate, tribasic monohydrate	1.84	1.45	1.75	1.85
Dicalcium phosphate	1.45	1.27	1.45	1.46
Clinton salt mix	1.11	1.11	1.11	1.12
Vitamin mix <sup>e</sup>	1.11	1.11	1.11	1.12
Calcium carbonate	0.61	0.61	0.61	0.62
Cholesterol	0.50	0.50	0.50	0.51
L-cystine	0.33	0.33	0.33	0.34
Choline bitrate	0.22	0.22	0.22	0.22
L-lysine	0.53	—	—	—
Blue dye	0.01	—	—	—
Purple dye	—	0.01	—	—
Red dye	—	—	0.01	0.02
Total	100	100	100	100

<sup>a</sup>Containing 45.7% carbohydrate, 42.1% fat, 17.8% protein on an as-fed basis.

<sup>b</sup>Containing 39.7% carbohydrate, 42.4% fat, 17.9% protein on an as-fed basis.

<sup>c</sup>Containing 39.5% carbohydrate, 42.5% fat, 18.0% protein on an as-fed basis.

<sup>d</sup>Containing 40.7% carbohydrate, 40.7% fat, 18.5% protein on an as-fed basis.

<sup>e</sup>Vitamin mix (g/kg) is from AIN-76A series (24).

ingredients were similar to other reported peanut amino acid concentrations (Young 1980). Vitamin free casein was used as the basic protein source for all diets and peanuts and peanut flour components were substituted in the respective diets based on the calculated protein content of each peanut component. Lysine was added into the whole peanut diet (0.5 g/100 g) because of the low amount present in the whole peanuts. Peanuts generally contain about 2 g/100 g of lysine and the peanuts used in this study contained only approximately 0.5 g/100 g of lysine. Lysine was not added to the FFPE diet because it was equivalent to 1 g/100 g after the removal of oil. The fatty acid profile of the oil in each individual diet is detailed in Table 2 since different fat types within saturation groups may have different atherogenic effects. The fatty acid profiles for the FFPE and control diets were similar but fatty acid profiles of the peanut oil and peanut diets were lower in saturated fatty acids and higher in unsaturated fatty acids. Tocopherol analysis indicated that all 4 hamster diets contained similar levels (data not presented).

All peanut based diets resulted in significantly lower TPC levels at 18 and 24 wk when compared to the control diet. TPC for animals consuming the peanut and peanut oil diets also had significantly lower TPC at 12 wk when compared to control, whereas TPC for hamsters consuming the FFPE did not differ significantly from the control (Table 3). TPC of hamsters fed any of the peanut based diets increased 3- to 4-fold from 0 to 24 wk, whereas TPC of hamsters fed control diets increased approximately 10-fold.

**Table 2—Fatty acid composition (%) in individual diets.**

Fatty acid	Diets			
	Fat free peanut flour	Peanut oil	Whole peanuts	Control
C 16:0	18.17	5.24	10.26	18.43
C 18:0	34.32	2.73	17.98	34.28
C 18:1	33.91	75.16	57.10	33.42
C 18:2	10.40	7.17	8.20	10.57
C 18:3	1.23	0.48	0.76	1.21
C 20:0	1.11	1.26	1.24	1.09
C 20:1	0.00	1.98	1.13	0.00
C 22:0	0.19	3.01	1.79	0.26
C 24:0	0.11	2.03	1.20	0.14

**Table 3—Total plasma cholesterol concentrations for peanut, peanut oil, fat free peanut flour, and control diet hamster groups at 0, 12, 18, and 24 wk.<sup>a</sup>**

Diet group	Time (wk)			
	0	12	18	24
	Total plasma cholesterol (mg/dL) $\pm$ SEM <sup>b</sup>			
Fat free peanut flour	105.9 $\pm$ 8.3 a	337.1 $\pm$ 58.8 ab	278.8 $\pm$ 152.3 b	445.6 $\pm$ 168.1 b
Peanut oil	105.9 $\pm$ 8.3 a	273.1 $\pm$ 42.4 bc	303.3 $\pm$ 55.7 b	360.7 $\pm$ 48.7 b
Whole peanuts	105.9 $\pm$ 8.3 a	236.9 $\pm$ 71.8 c	294.4 $\pm$ 121.8 b	325.6 $\pm$ 99.6 b
Control	105.9 $\pm$ 8.3 a	418.9 $\pm$ 161.7 a	535.2 $\pm$ 123.0 a	1081.6 $\pm$ 340.5 a

<sup>a</sup>Numbers in columns followed by the same letter are not significantly different ( $P < 0.05$ ).

<sup>b</sup>SE of the mean.

**Table 4—Very low density lipoprotein cholesterol concentrations for peanut, peanut oil, fat free peanut flour, and control diet hamster groups at 0, 12, 18, and 24 wk.<sup>a</sup>**

Diet group	Time (wk)			
	0	12	18	24
	Very low density lipoprotein cholesterol (mg/dL) $\pm$ SEM			
Fat free peanut flour	33.4 $\pm$ 4.4 a	89.4 $\pm$ 30.0 a	90.7 $\pm$ 61.8 ab	183.0 $\pm$ 124.3 b
Peanut oil	33.4 $\pm$ 4.4 a	36.7 $\pm$ 12.1 b	50.8 $\pm$ 17.3 b	66.0 $\pm$ 36.1 b
Whole peanuts	33.4 $\pm$ 4.4 a	44.2 $\pm$ 20.1 b	90.4 $\pm$ 63.6 ab	122.3 $\pm$ 69.8 b
Control	33.4 $\pm$ 4.4 a	96.5 $\pm$ 59.9 a	154.2 $\pm$ 62.3 a	553.7 $\pm$ 338.6 a

<sup>a</sup>Numbers in columns followed by the same letter are not significantly different ( $P < 0.05$ ).

A blood lipid profile high in VLDL-C and LDL-C indicates a higher risk of CVD. Peanut components appear to have prevented an increase in non-HDL-C (Table 4 and 5) concentrations in hamsters from wk 12 to 24. VLDL-C (Table 4) trends were similar to that of LDL-C. Data in Table 5 indicate that at 18 wk all peanut component diet hamsters had significantly lower LDL-C concentrations than those consuming the control diet. At 24 wk, LDL-C concentrations in hamsters in the FFPE, peanut oil, and whole peanut diet groups did not differ significantly while each peanut diet group did differ significantly from the control. Compared to hamsters that consumed peanut component diets, the control diet produced a significant increase in VLDL-C concentrations between wk 12 and 24 ( $P = 0.0442$ ) and again between wk 18 and 24 ( $P = 0.0002$ ) (Table 4). All peanut component diets had a significantly lower rate of VLDL-C and LDL-C increase between wk 0 and 24 compared to the control diet.

To reduce the risks for CVD, a high plasma HDL-C concentration is as important as a low LDL-C cholesterol concentration (Gordon and others 1989). Results (Table 3, 4, and 5) indicate that after 18 wk, TPC, VLDL-C, and LDL-C were all significantly lower in animals fed the peanut and peanut component diets. However, HDL-C concentrations were not significantly different among any of the peanut diets or the control diet (Table 6). There was not a time effect on the rate of increase of HDL-C concentration in any of the diet groups.

Chemical analyses for TC and CE were performed on extracted hamster aortas. This analysis method was adopted when the microscopic examination of Oil Red O stained aortic tissue from 24 wk control diet hamsters did not reveal visible aortic lesions. The aortas that were stained were not chemically analyzed so data for TC and CE are from time points 0, 12, and 18 wk only. Aortic TC was significantly lower in the FFPE and peanut oil diet groups than the control and whole peanut groups at wk 12. Aortic TC in all diet groups increased from 12 to 18 wk with the peanut component diet groups increasing by approximately 52 to 152 mg/g PR and the control diet group increasing by approximately 442 mg/g PR. Concentrations of aortic TC in the animals consuming the control diet were significantly higher and generally almost numerically double the TC concentrations in the peanut diet groups at 18 wk (Table 7).

Aortic CE increased from 0 to 12 wk in all diet groups and at 12 wk differences in CE concentration among the 4 diet groups were not significant. However, at wk 18, CE concentrations in animals consuming any of the peanut component diets were significantly lower than the CE concentration in animals consuming the control diet. The CE concentration in aortas of animals consuming the control diet increased by 15.4 mg/g PR between wk 12 and 18 while in the peanut diet groups the increases were 1.6 in whole peanuts, 2.1 in FFPE, and 5.9 in the peanut oil diet group.

## Discussion

The present study was designed to investigate the effects of peanuts and peanut components on blood chemistry CVD risk factors and the incidence of atherosclerosis in hamsters consuming a high fat, high cholesterol diet. Each diet was intended to be isocaloric. Because the FFPE ingredient contained less than 0.5% fat, there was minimal fat substitution within the AIN-76A Clinton/Cybulsky (control) diet for rodents during formulation of the FFPE experimental diet. The peanut oil diet had the greatest amount of fat substitution compared to the control diet. These substitutions resulted in a high level of cocoa butter in the FFPE diet compared to the peanut oil and peanut diets which resulted in dissimilar fatty acid profiles within the diets (Table 2). Peanut oil and peanut diets were higher in monounsaturated fatty acids (MUFA) with 16.6 and 12.3 g/100g, respectively; while the control and FFPE diets had 6.6 and 6.4 g/100g of MUFA, respectively. Polyunsaturated fatty acids (PUFA) were similar among all diets with the control diet having 2.4 g/100g; while the FFPE, peanut oil and whole peanut diets had 2.3, 1.7, and 2 g/100g, respectively. Saturated fat varied in the experimental diets. The peanut oil diet contained the lowest amount of saturated fat with 3.6 g/100g, while the FFPE diet had 11.7 g/100g, which was similar to the control diet at 12.3 g/100g. The different fatty acid profiles may have influenced the rate of increase in blood chemistry risk factors in the earlier time points. TPC and LDL-C in the FFPE diet and the control diet were not statistically different at 12 wk possibly due to the similarity of their fatty acid profiles. The fatty acid in the peanut oil diet was mainly (75.16%) oleic acid, which has been linked with reductions in hypertension (Terés and others 2008). The peanut oil diet was also very low in stearic acid (2.73%), while the control and FFPE diets

contained much higher levels (approximately 34.3%). Results from the peanut oil and peanut diets are in agreement with the body of literature indicating that replacement of saturated fatty acids with oleic acid or linoleic acid lowers serum cholesterol levels (Hooper and others 2001). Fatty acid profiles which may have influenced the rate of change in blood chemistry risk factors apparently did not influence the rate of change in aortic TC or CE (Table 7) and may suggest involvement of different mechanisms for changes of these different factors by each diet. The mechanisms are not known but it is clear that the non-lipid portion, and not the fatty acid profile, of the FFPE diet had a positive effect on CVD risk factors and the development of atherosclerosis.

After 12 wk of consuming high fat, high cholesterol diets, TPC in all diet groups was equal or higher than the published normal diet range for TPC (91 to 237 mg/dL) (Campbell 2004) for hamsters. This could be interpreted to indicate that peanuts had little effect on the initial increase in TPC but affected the rate after the initial increase. Alternatively, the data may simply be interpreted to mean that this particular strain of hamsters has a generally higher "normal" TPC range than hamsters on which the earlier data were based. Studies are currently underway to examine, not only, additional peanut related effects on blood risk factors with a high fat, high cholesterol diet, but also, to determine the normal levels of these risk factors over time in male Syrian golden consuming a standard commercial hamster diet.

Except at 12 wk, as noted previously, when the FFPE diet was not significantly different from the control, the peanut and peanut component diets resulted in consistently statistically lower TPC than in the control diet (Table 3). From wk 12 to 24 the TPC concentration in control group hamsters doubled to 1081.6 mg/dL while the TPC levels of the FFPE, peanut oil and whole peanut groups only increased by 96.8, 57.8, and 31.2 mg/dL, respectively.

The reduction of LDL-C concentration with the peanut and peanut oil diets is consistent with other published data (Kris-Etherton and others 1999b). The peanut oil and peanut groups consumed diets containing the highest level of MUFA, which have demonstrated to significantly reduce LDL-C concentrations. The FFPE diet had a significantly lower amount of MUFA than the peanut oil and whole peanut diets; however, at the end of the study, the results for LDL-C were not significantly different among the

**Table 5 – Low density lipoprotein cholesterol concentrations for peanut, peanut oil, fat free peanut flour, and control diet hamster groups at 0, 12, 18, and 24 wk.<sup>a</sup>**

Diet group	Time (wk)			
	0	12	18	24
	Low density lipoprotein cholesterol (mg/dL) $\pm$ SEM			
Fat free peanut flour	15.4 $\pm$ 4.4 a	114.1 $\pm$ 40.2 a	110.3 $\pm$ 71.6 b	141.4 $\pm$ 101.6 b
Peanut oil	15.4 $\pm$ 4.4 a	21.8 $\pm$ 20.3 b	20.3 $\pm$ 7.9 c	60.2 $\pm$ 41.3 b
Whole peanuts	15.4 $\pm$ 4.4 a	9.2 $\pm$ 5.9 b	27.8 $\pm$ 28.3 bc	47.5 $\pm$ 37.6 b
Control	15.4 $\pm$ 4.4 a	143.9 $\pm$ 133.2 a	224.5 $\pm$ 88.1 a	354.3 $\pm$ 91.8 a

<sup>a</sup>Numbers in columns followed by the same letter are not significantly different ( $P < 0.05$ ).

**Table 6 – High density lipoprotein cholesterol concentrations in peanut, peanut oil, fat free peanut flour, and control diet hamster groups at 0, 12, 18, and 24 wk.<sup>a</sup>**

Diet group	Time (wk)			
	0	12	18	24
	High density lipoprotein cholesterol (mg/dL) $\pm$ SEM			
Fat free peanut flour	57.0 $\pm$ 7.9 a	173.6 $\pm$ 17.2 a	147.8 $\pm$ 31.5 b	150.0 $\pm$ 81.0 a
Peanut oil	57.0 $\pm$ 7.9 a	217.5 $\pm$ 28.4 a	232.1 $\pm$ 38.6 a	225.2 $\pm$ 61.1 a
Whole peanuts	57.0 $\pm$ 7.9 a	183.5 $\pm$ 52.2 a	176.2 $\pm$ 36.9 b	155.8 $\pm$ 62.9 a
Control	57.0 $\pm$ 7.9 a	178.5 $\pm$ 35.1 a	156.5 $\pm$ 18.5 b	163.6 $\pm$ 125.3 a

<sup>a</sup>Numbers in columns followed by the same letter are not significantly different ( $P < 0.05$ ).

**Table 7 — Total aortic cholesterol and aortic cholesteryl ester concentrations in peanut, peanut oil, fat free peanut flour, and control diet hamster groups at 0, 12, and 18 wk.<sup>a,b</sup>**

Diet group	Total cholesterol			Cholesteryl ester		
	time (wk)			time (wk)		
	0	12	18	0	12	18
	mg/g protein $\pm$ SEM			mg/g protein $\pm$ SEM		
Fat free peanut flour	1.3 $\pm$ 0.1	304.4 $\pm$ 2.5 b	456.6 $\pm$ 2.6 b	0.4 $\pm$ 0.1	1.6 $\pm$ 1.1 a	3.7 $\pm$ 1.1 b
Peanut oil	1.3 $\pm$ 0.1	292.5 $\pm$ 1.7 b	390.5 $\pm$ 4.3 b	0.4 $\pm$ 0.1	1.7 $\pm$ 0.9 a	7.6 $\pm$ 1.4 b
Whole peanuts	1.3 $\pm$ 0.1	350.0 $\pm$ 2.0 a	402.5 $\pm$ 2.6 b	0.4 $\pm$ 0.1	2.4 $\pm$ 1.2 a	4.0 $\pm$ 1.0 b
Control	1.3 $\pm$ 0.1	407.7 $\pm$ 3.2a	849.0 $\pm$ 6.9a	0.4 $\pm$ 0.1	2.8 $\pm$ 2.6a	19.2 $\pm$ 2.9a

<sup>a</sup>TC means in columns followed by the same letter are not significantly different ( $P < 0.05$ ).

<sup>b</sup>CE means in columns followed by the same letter are not significantly different ( $P < 0.0001$ ).

peanut diets although all were significantly different from the control diet (Table 5).

The FFPE diet contained the highest amount of fiber, about 6.6%, the lowest amount was in the whole peanut diet with about 4.1%, and the peanut oil and control diets contained 5.8% and 5.6%, respectively. High levels of fiber have been indicated to reduce plasma LDL-C concentrations (Jayakumari and Kurup 1979; Pellizzon and others 2007). The fiber content of the individual diets in this study were slightly different but because the results consistently indicated that peanut diets had positive effects on blood chemistry risk factors, overall, the data suggest that fiber content did not affect the observed results. Data indicating that FFPE had similar CVD risk factor and atherosclerosis effects as whole peanuts and peanut oil have not been previously reported. These data indicate that nonlipid components have significant CVD protective effects. Arginine, flavonoids, and folates are all found in peanuts and these components have been linked to cardiovascular health (Ward and others 1997; Kris-Etherton and others 1999b; Wells and others 2005; Mink and others 2007). The concentrations of these and other components were not determined in FFPE or the other diets. However, along with the high concentration of peanut protein, these and other components may have contributed to the mechanism resulting in lower LDL-C concentrations (compared to control) in animals consuming the peanut and FFPE diets. The peanut oil diet is assumed to not contain protein or folates; however, the collective data for the peanut and peanut component diets suggest that both lipid and non-lipid components in peanuts affect CVD risk factors and atherosclerosis development even though the effects in the peanut oil and FFPE diets were not found to be additive in the peanut diet. Additional research is needed to ascertain the role of specific nonlipid components in the effects determined in this study.

All diets were isocaloric and contained about 4.5 kcal/g. Daily feed intake was approximately 14 g for a total of 63 kcal per day. Each peanut and peanut component diet contained 20% by weight of the individual peanut components. The 4.5 kcal/g of each diet was derived from different components in the diets including the differential caloric contribution from added peanuts, peanut oil and FFPE. Some nutritional studies incorporate very high levels of components to elicit a particular response in animals. A comparison of hamster consumption of peanut oil and FFPE to common human consumption is not logical because those products are not consumed in that form. However, a caloric comparison with human consumption can be made for the peanut diet. The caloric load of roasted peanuts is about 5.6 kcal/g so hamsters on the peanut containing diet obtained 15.7 kcal per day from peanuts (20% peanuts in the feed  $\times$  14 g of diet consumed per day  $\times$  5.6 kcal/g from peanuts = 15.7 kcal). Hamster calorie consumption from peanuts in the peanut diet would thus be about 25%. A consumer serving of

peanuts (28.4 g) is approximately 160 kcal. A sedentary (light physical activity associated with typical day-to-day life) human on a 2000 kcal diet (AHA 2010) would have to consume about 498 kcal or 3.1 servings of peanuts per day for an equivalent caloric intake from peanuts. Consumer consumption of 3.1 servings (87.9 g) would be considered high on a regular basis but occasional daily consumption of that amount is not unusual in a portion of the general population. Intake levels of peanuts and peanut components producing the responses identified were appropriate for an animal study although slightly higher than normal human consumption levels. However, the fact that all diets resulted in the same average weight gain and the FFPE, peanut oil, and peanut diets generally resulted in similar blood risk factors and CE levels suggests that the caloric contribution of peanut ingredient had little effect on the results obtained and alternatively suggests that the results were possibly related to particular peanut components present in those diets and absent in the control diet. Human epidemiological studies (Fraser and others 1992; Hu and others 1998; Alper and Mattes 2003) have demonstrated that regular consumption of a serving of nuts, including peanuts, resulted in a significant reduction in CVD risk so reduced peanut levels may have produced the same results as 25% of calories from peanuts.

CE analysis has been highly correlated to the development of atherosclerosis and used as an indicator for atherosclerosis in previous studies (Chobanian and Manzur 1972; Day and Proudlock 1974; Kerckhoffs and others 2002) because it is one of the first metabolic compounds associated with the development of atherosclerosis (St. Clair 1976). At wk 18 all hamsters that consumed diets containing peanut components had significantly lower aortic TC and CE compared to hamsters consuming the control diet (Table 7). The FFPE, peanut oil, and peanut diet groups had a significantly lower rate of CE increase as determined from the slope of a best fit line for the 0 to 18 wk (data not shown). Data in Table 7 indicates that the rate of increase in CE was essentially the same in all diet groups through 12 wk. However, CE in the control diet group continued to increase in the same linear manner from 12 to 18 wk as from 0 to 12 wk. CE increases in the peanut diet groups did not continue in the same linear manner from 12 to 18 wk as from 0 to 12 wk which resulted in significant differences in CE between the control and all peanut diet groups at 18 wk.

## Conclusions

Results demonstrated that FFPE, as well as peanuts and peanut oil, reduced TPC and non-HDL-C without reducing HDL-C cholesterol levels in hamsters when added to a diet known to induce atherosclerosis. The results are consistent with published data in that peanuts and peanut oil resulted in reduced risk factors for CVD. However, this study is the 1st report of a non-lipid peanut component positively impacting CVD risk factors. In addition, the

results demonstrated that peanuts, peanut oil and FFPF retarded the increase of aortic CE, a primary metabolic parameter associated with the development of atherosclerosis, and suggest that peanuts, peanut oil, and FFPF retard the development of atherosclerosis in a diet designed to promote atherosclerosis. These results are supported by published epidemiological studies that demonstrated that regular consumption of nuts, including peanuts, resulted in reduced CVD in humans.

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